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10/620,794	07/15/2003	Jeff J. Staggs		1100

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EXAMINER	
WEDDINGTON, KEVIN E	

ART UNIT	PAPER NUMBER
1614	

DATE MAILED: 10/13/2005

Please find below and/or attached an Office communication concerning this application or proceeding.

Interview Summary	Application No.	Applicant(s)	
	10/620,794	STAGGS, JEFF J.	
	Examiner	Art Unit	
	Kevin E. Weddington	1614	

All participants (applicant, applicant's representative, PTO personnel):

(1) *Kevin E. Weddington*. (3)_____.

(2) *Jeff J. Staggs*. (4)_____.

Date of Interview: *05 October 2005*.

Type: a)☒ Telephonic b)☐ Video Conference
c)☐ Personal [copy given to: 1)☐ applicant 2)☐ applicant's representative]

Exhibit shown or demonstration conducted: d)☐ Yes e)☒ No.
If Yes, brief description: _____.

Claim(s) discussed: *The claims in general.*.

Identification of prior art discussed: *Yamaguchi et al*.

Agreement with respect to the claims f)☒ was reached. g)☐ was not reached. h)☐ N/A.

Substance of Interview including description of the general nature of what was agreed to if an agreement was reached, or any other comments: *The applicant of record, Jeff J. Staggs, stated that he did not received and Yamaguchi et al. reference in the mailed Office action dated June 1, 2005. The Examiner called the applicant on October 5, 2005, and the applicant stated that he would like the Yamaguchi et al. reference to be mailed to him. The applicant, Mr. Staggs, also stated he may called the Examiner to give a new fax number for future faxes*.

(A fuller description, if necessary, and a copy of the amendments which the examiner agreed would render the claims allowable, if available, must be attached. Also, where no copy of the amendments that would render the claims allowable is available, a summary thereof must be attached.)

THE FORMAL WRITTEN REPLY TO THE LAST OFFICE ACTION MUST INCLUDE THE SUBSTANCE OF THE INTERVIEW. (See MPEP Section 713.04). If a reply to the last Office action has already been filed, APPLICANT IS GIVEN ONE MONTH FROM THIS INTERVIEW DATE, OR THE MAILING DATE OF THIS INTERVIEW SUMMARY FORM, WHICHEVER IS LATER, TO FILE A STATEMENT OF THE SUBSTANCE OF THE INTERVIEW. See Summary of Record of Interview requirements on reverse side or on attached sheet.

K. Weddington

Kevin E. Weddington
Primary Examiner
Art Unit 1614

Examiner Note: You must sign this form unless it is an Attachment to a signed Office action.

Examiner's signature, if required

[Bull. Tokyo Kasei Daigaku 25, p. 201~203, 1985]

Antibacterial and Antitumor Activities of Piperine from Black Pepper

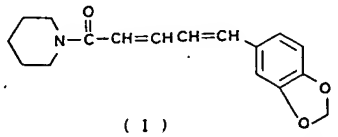
Isao YAMAGUCHI and Sachiko OZEKI

(Received October 9, 1984)

Introduction

Piperine (I) was isolated from black pepper (Piper nigrum L.) as long ago as 1820, later also from other pepper fruits such as P. longum L., P. retrofructum Vahl (P. officinarum C. DC.), and P. clusii C. DC., from root bark of P. geniculatum Sw. and Piperaceae.

Oerstedt[1] (1821) suggested that the presence of piperine gave the pungency of black pepper but later it was clarified by Buchheim[2] (1876) that chavicine which was the stereoisomer of piperine had more pungent taste than piperine, and the structure of piperine was proven by Ladenburg and Scholtz[3] (1894).



(1)

Harvill[4] et al (1943) showed that piperine was more toxic than pyrethrum against housefly but according to Su[5] (1977), piperine was not the constituent in black pepper that was responsible for contact texicity to the insects.

Salzer[6] et al (1977) showed that pepper was active against Eschericia coli in sausage but they found the curing organisms, the micrococci and lactobacilli, to be unaffected by relatively high concentrations. Hitokoto[7] et al (1978) reported that the chloroform extract of black peper fruits powder had from 1 to 7% inhibition of the growth and 100% inhibition of the toxin production of several toxigenic fungi. Huhtanen[8] (1980) also showed that the ethanol extract of black pepper was active against Clostridium botulinum with a minimum inhibitory concentration ($\mu g/ml$) of 125 ppm.

From last three literatures we did not know what constituent was active against them, so we reported in this paper the results of bioassay for antibacteria and antitumor activites of piperine, of all constituents from black pepper.

Experimental and Results

Dried black pepper fruits from Brazil in 1980 were presented for us by Takasago Perfumery Co. Piperine was purified from the chloroform extract in our laboratory, all solvents used for extraction and recrystalization were distilled once before use and of reagent grade quality commercially in Japan.

The infrared spectrum was taken by a JEOL IRA-1 spectrometer, the nuclear magnetic resonance spectrum for ^1H was recorded by a Hitachi R-40 90 MHz spec-

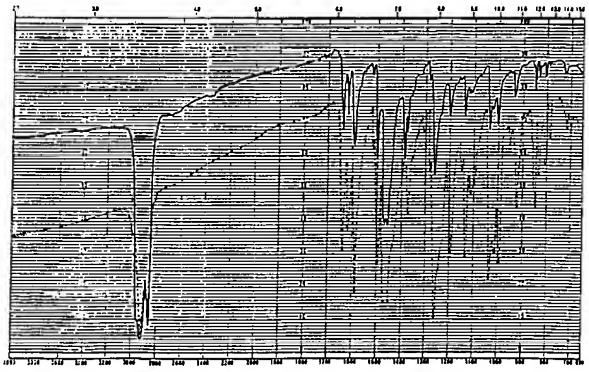


Fig. 1. IR Spectrum of Piperine from Black Pepper (nujol mull) (solid line), the Standard Spectrum of Piperine (nujol mull) (broken line)

The 3rd Laboratory of Nutrition

Tokyo Kasei Daigaku, Tokyo

trometer using tetramethylsilane as an internal reference and the melting point was measured with a Meiho automatic thermal analyser MR-2.

The bioassays of piperine for bactericidal and antitumor activities were proceeded by the laboratory of Kyowa Hakko Kogyo Co.

1. Extraction and Purification of Piperine

The extract was made by steeping 5 kg of dried black pepper fruits in 6 *l* of chloroform after benzene at room temperature with occasional stirring, the extract was filtered with a filter paper and the filtrate was concentrated continuously with a rotary evaporator into a

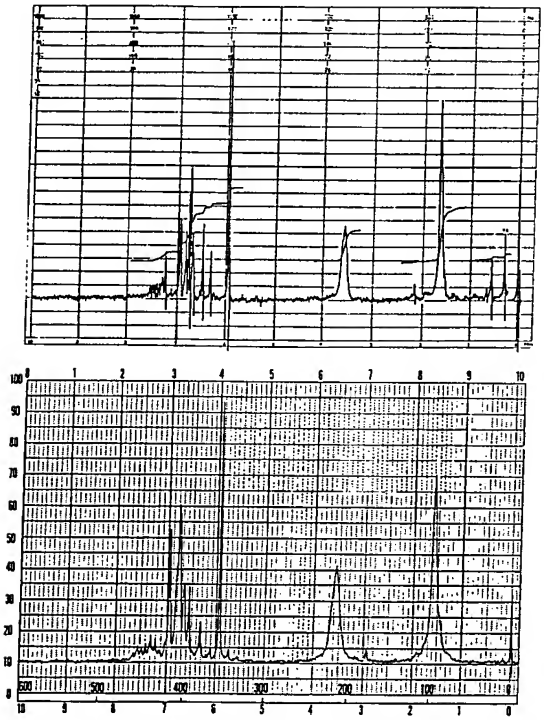


Fig. 2. ^{1}H-NMR Spectrum of Biperine from Black Pepper (above), the Standard Spectrum of Piperine (below)

paste. 50.6g of the residue was separated to 9 fractions by a silica gel (Kiesel Gel 60 Merck) column (5.0 x 45.0 cm) chromatography eluted with solvents of benzene: ethyl accetate (4:1 v/v), acetone and n-butanol by turns, the fraction 4 of benzene:ethyl acetate was concentrated with a rotary evaporator, 1.45g of monoclinic prisms (like rock sugers) was obtained after recrystalization with benzene from 4.5g of the residue. m.P. 131°C (L[9]. 130°C), the tlc showed one spot at Rf=0.44, which was developed with benzene:acetone (9:1 v/v). From the data of the infrared spectrum and the ^{1}H-nmr spectrum, the prisms was identified piperine in comparison with the authentic spectra[11,12] (Fig. 1 and 2).

2. Bioassay of Piperine against Bacteria

Piperine was bioassyed in vitro against 27 species of bacteria, from 10 genus mainly enterobacteriaceae. The results showed that piperine was active with a MIC of 100 ppm against Pseudomonus auruginosa # 1 and Alcaligenes F 2518 (Table 1). Determination of MIC was made visually by observing turbidity with a photometer detected through transmitted light.

Table 1. Antibacterial Activities of Piperine in Vitro

Species	MIC(μg/ml)
Staphylococcus aureus 209-P	>100
S. aureus SMITH	> 100
S. epidermidis	> 100
Escherichia coli NIHJJC-2	> 100
E. coli CN2411-5	> 100
E. coli JUHL	> 100
Klebsiella pneumoniae 3045	> 100
K. pneumoniae Y-60	> 100
Serratia marcescens T-26	> 100
S. marcescens T-55	> 100
Proteus mirabilis 1287	> 100
P. vulgaris 6897	> 100
P. morganii KY4298	> 100
P. rettgeri 4289	> 100
Enterobactor cloaceae F1510	> 100
E. cloaceae F1870	> 100
E. aerogenes F1948	> 100
E. aerogenes F1949	> 100
Citrobactor freundii F1526	> 100
C. freundii F1528	> 100
Pseudomonas aeruginosa #1	100
P. aeruginosa DBT145	> 100
P. putida F264	> 100
P. cepacia F2251	> 100
P. maltophilia F3438	> 100
Acinetobactor F2575	> 100
Alcaligenes F2518	100

3. Bioassay of Piperine against Sarcoma-180A Solid Tumor

Piperine was also bioassayed against the intraperitoneal sarcoma-180A solid tumor in mice. The results revealed that the activity of piperine was no match for mitomycin C which was developed by Kyowa Hakko Kogyo Co., Japan (Table 2). Determination of activity was made by comparing the weight of tumor untreated with the weights of tumor treated with drugs injected intraperitoneally.

Table 2. Anti-Solid-Tumor Activity of Piperine against Sarcoma-180A in Mice

Drug	Dose(mg/kg)	Treated/Control(wt)
Control	—	— (15.37㎎)
Mitomycin C	6× 1 (i.p)	0.48
Piperine	100× 1 (i.p)	0.77
	400× 1 (i.p)	0.72

Discussion

We knew since old times that pepper fruits powder was sprinkled over meat for curing. It was reasonable scientifically because a certain component had inhibition of the growth and the toxin production of toxigenic funji, and was active against Clostridium botulinum. Piperine also had activities against Ps. aeruginosa #1 and Alcaligenes F2518. Pillitorine which was one of Piperaceae amides had activity against Lows lung carcinoma in mice Loder[10] et al (1969), but piperine had not responsibility for the sarcoma-180A tumor in mice.

Finally we express our appreciation to staffs of the laboratory of Kyowa Hakko Kogyo Co. for bioassays of piperine, to Misses Sayuri Chiba and Michiko Natsume who were students in our laboratory for extractions with several solvents..

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黒こしょうからのピペリンの抗細菌性と抗腫瘍性

山口　功・尾関幸子

（昭和59年10月9日受理）

黒こしょうから抽出して得たピペリンについて抗細菌活性を調べた結果，Pseudomonas, aeruginosa #1（緑膿菌）と Alcaligenes F2518（腸内および酪農産物細菌）に活性を示したが，抗腫瘍性試験では Sarcoma-180A固型腫瘍に対して，市販の抗癌剤であるマイトマイシンCに比し約1／27の活性しか示さなかった．